

AMENDMENTS TO THE CLAIMS

Claim 1 (currently amended)

A protein of fungal origin having epoxide hydrolase activity, ~~such as is obtained~~ in ~~essentially~~ pure form by extraction from cells of fungi, or by culture of host cells transformed by a nucleotide sequence coding for the aforementioned fungal protein ~~or protein derived by substitution, suppression or addition of one or more amino acids of the~~ aforementioned protein of fungal origin and possessing epoxide hydrolase activity.

Claim 2 (currently amended)

A protein of claim 1, comprising sequence SEQ ID No: 2,

~~or any sequence derived from sequence SEQ ID No: 2, by substitution, suppression or addition of one or more amino acids, and possessing epoxide hydrolase, the said derived sequence having a homology of at least about 40% with sequence SEQ ID No: 2,~~

~~or any fragment of sequence SEQ ID No: 2, or of a sequence derived from the latter as defined above, and possessing epoxide hydrolase activity, the said fragment consisting of at least about 10 amino acids that are contiguous in the region delimited by the amino acids located in positions 1 and 339 of the sequence SEQ ID No: 2.~~

Claim 3 (currently amended)

A protein of claim 1, corresponding to a fungal epoxide hydrolase in ~~essentially~~ pure form, as obtained by extraction and purification from cultures of cells of fungi of the *Aspergillus* species.

Claim 4 (currently amended)

A protein of claim 1 corresponding to the fungal epoxide hydrolase in ~~essentially~~ pure form represented by SEQ ID No: 2, obtained by extraction and purification from cultures of cells of strains of the *Aspergillus niger* or *Aspergillus turingensis*.

Claim 5 (currently amended)

A protein of claim 1 corresponding to a recombinant fungal epoxide hydrolase, as obtained in ~~essentially~~ pure form by transformation of ~~suitable~~ host cells by means of vectors containing:

- the nucleotide sequence SEQ ID No: 1 encoding the epoxide hydrolase of SEQ ID No: 2, ~~or any sequence derived from SEQ ID No: 1 by degeneration of the genetic code, and encoding the epoxide hydrolase represented by SEQ ID No: 2,~~

~~or any sequence derived from the sequence SEQ ID No: 1, by substitution, suppression or addition of at least one nucleotide, and coding for an enzyme possessing epoxide hydrolase activity, the said derived sequence having a homology of at least about 45% with the sequence SEQ ID No: 1,~~

~~or any fragment of the sequence SEQ ID No: 1, or of a sequence derived from the latter as defined above, and coding for an enzyme possessing epoxide hydrolase activity, the said fragment consisting of at least about 20 nucleotides that are contiguous in the region delimited by the nucleotides located in positions 1 and 1197 of the sequence SEQ ID No: 1.~~

Claim 6 (currently amended)

A protein of claim 5, corresponding to the fungal recombinant epoxide hydrolase represented by SEQ ID No: 2 as obtained by transformation of suitable host cells by vectors containing the nucleotide sequence SEQ ID No: 1, ~~or any sequence derived from SEQ ID No: 1 by degeneration of the genetic code,~~ and encoding the epoxide hydrolase represented by SEQ ID N.: 2.

Claim 7 (previously presented)

A nucleotide sequence encoding a protein of fungal origin with epoxide hydrolase activity as defined by claim 1.

Claim 8 (currently amended)

A nucleotide sequence according to claim 7, characterized in that it comprises:

- the sequence represented by SEQ ID No: 1 encoding the epoxide hydrolase represented by SEQ ID No: 2,

- or any sequence derived from the sequence SEQ ID No: 1 by degeneration of the genetic code, and encoding the epoxide hydrolase represented by SEQ ID No: 2,

- or any sequence derived from the sequence SEQ ID No: 1, ~~especially~~ by substitution, suppression or addition of one or more nucleotides, and coding for an enzyme possessing epoxide hydrolase activity, the said derived sequence ~~preferably~~ having a homology of at least about 45% with the sequence SEQ ID No: 1,

- or any fragment of the sequence SEQ ID No: 1, or of a sequence derived from the latter as defined above, and coding for an enzyme possessing epoxide hydrolase activity, the said fragment preferably consisting of at least about 20 nucleotides that are contiguous in the region delimited by the nucleotides located in positions 1 and 1197 of the sequence SEQ ID No: 1,

- or any complementary nucleotide sequence of the aforementioned sequences or fragments,

- or any nucleotide sequence coding for an enzyme possessing epoxide hydroloase activity, and capable of hybridization with one of the aforementioned sequences or fragments,

- the aforementioned sequences or fragments being of single-stranded or double-stranded form.

Claim 9 (currently amended)

A vector, ~~especially a plasmid~~, containing a nucleotide sequence according to claim 7.

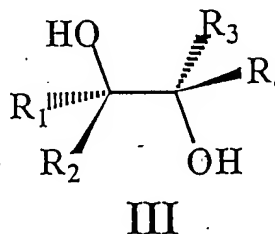
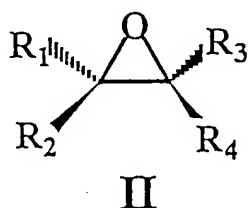
Claim 10 (currently amended)

A host cell selected from the group consisting of bacteria, viruses, yeasts, fungi, plants and mammalian cells, the said host cell being transformed by a vector of claim 9 so that its genome contains a nucleotide sequence of encoding a protein of fungal origin with epoxide hydrolase activity.

Claim 11 (cancelled)

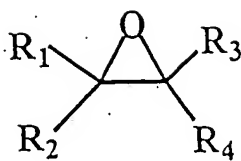
Claim 12 (currently amended)

A method of preparation of epoxides and/or of enantiomerically pure diols respectively of the following formulae



in which R^1 , R^2 , R^3 and R^4 represent any group characteristic of pharmaceutical and plant-protection compounds, or of specific optical materials forms corresponding to the said epoxides or vicinal diols of formulae II and III

comprising treating a mixture of diastereoisomeric epoxides, or of a chiral epoxide in racemic form, or of a prochiral epoxide of the formula:



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with a protein of fungal origin having epoxide hydrolase activity, obtained in pure form by extraction from cells of fungi, or by culture of host cells transformed by a nucleotide sequence coding for the aforementioned fungal protein and with epoxide hydrolase activity ~~of claim 1~~ or with the host cell of claim 10 expressing a protein of fungal origin having epoxide hydrolase activity, obtained in pure form by extraction from cells of fungi, or by culture of host cells transformed by a nucleotide sequence coding for the aforementioned fungal protein and with epoxide hydrolase ~~of claim 1~~, which leads to the production of:

- a mixture of the aforementioned compounds of formula (II) and (III), and optionally separating the aid compounds of formulae (II) and (III) by an additional stage of purification,
- or ~~of just~~ only the aforementioned compound of formula (III).

Claim 13 (previously presented)

A method of preparation of a protein with recombinant epoxide hydrloase activity of claim 5 comprising transforming host cells selected from the group consisting of bacteria, viruses, yeasts, fungi, plants and mammalian cells with a vector of claim 9, and

purifying the recombinant epoxide hydrolase produced by the said cells.

Claim 14 (currently amended)

A method of preparation of a protein with epoxide hydrolase activity in essentially pure form of claim 3 comprising:

- extracting the enzyme from cellular cultures of fungi by crushing the fungus using a press, followed by low-speed centrifugation, recovery of the supernatant, and optionally, concentration of the same,
- and purifying the enzyme from the extract obtained in the preceding stage by successive passages through columns of DEAE-Sepharose, Phenyl-Sepharose, Mono Q and Superose 12.

Claim 15 (previously presented)

In the process of preparation of epoxides or enantiomerically pure vicinal diols with enzymatic biocatalysts, the improvement comprising using as the biocatalyst a protein of claim 1 with epoxide hydrolase activity.